

ON THE ENERGETICS OF DIFFERENTIATION

II. A COMPARISON OF THE RATES OF DEVELOPMENT OF GIANT AND OF NORMAL SEA-URCHIN EMBRYOS

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The following experiments show that normally proportioned giant embryos produced by the fusion of two fertilized eggs develop more rapidly than do normal embryos. This result had been anticipated (Tyler, 1933) on the basis of some experiments on the oxygen consumption and rate of development of half-sized embryos.

Theoretical Part

It has been shown (Tyler, 1933) that the rate of oxygen consumption of two half-sized embryos from isolated blastomeres is the same as that of one normal embryo up to the gastrula stage. The dwarf embryos, however, develop more slowly than the normal. Thus, when compared at the same stage of development (e.g., end of gastrulation), the total oxygen consumption of two dwarf embryos is greater than that of a single normal embryo. The theory upon which these results are interpreted involves the following assumptions: (1) Work is performed (and energy thus required) in the process of gastrulation as in other form changes in embryonic differentiation. (2) The amount of work performed (or energy required) varies directly with the amount of material involved and inversely with some function of the radius of curvature of the embryo or of the part considered. (3) The oxygen consumption is a measure of the energy liberated.

On the basis of assumptions 1 and 2, two dwarf embryos from isolated blastomeres should perform more work (and thus require more energy) for the process of gastrulation than one normal embryo. The experiments show that two dwarf embryos have the same rate of oxygen consumption as one normal embryo. On the basis of assumption 3 this means that energy is liberated at the same rate in the two dwarf embryos as in the normal embryo. The slower development of the dwarf embryo was thus accounted for, since with half the

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amount of energy available per unit time and with more than half the amount of work to be performed, more time is required to supply the amount of energy necessary for the various processes.

These considerations led to the expectation that giant embryos produced by the fusion of two fertilized eggs should develop faster than the normal embryos.

Experimental Part

Methods of fusing eggs have been described by Morgan (1895), Driesch (1900), Bierens de Haan (1913), Goldfarb (1913, 1915), von Ubisch (1925), Hörstadius (1928) and Balinsky (1932) for sea urchins and by Mangold (1920) and Mangold and Seidel (1927) for salamanders.

Morgan simply removed the membrane shortly after fertilization and found that in a concentrated mass some eggs would fuse in pairs. Driesch treated the eggs with Ca-free sea water after removal of the fertilization membrane and also tried alkaline sea water and centrifuging. Bierens de Haan used essentially the same method. Goldfarb placed eggs in an isotonic or hypotonic solution of NaCl in sea water for considerable lengths of time. Von Ubisch allowed the eggs to remain in Ca-free sea water from the two to four-cell stage, and then brought them close together in ordinary sea water. Hörstadius fused groups of blastomeres with other groups of blastomeres or with whole eggs by bringing them together in a groove in a piece of cinema film in a dish of Ca-free sea water. Balinsky used the same method for fusing two whole eggs in the four-cell stage.

Most of the various methods of fusion were tried. In all the experiments the membranes are first removed either by shaking the eggs in a tube or sucking them up in a pipette one to three minutes after fertilization. If the eggs are fairly concentrated they tend to stick together after removal of the membrane in clumps of various numbers, as noted by Morgan (1895). The eggs are apparently capable of sticking to one another during the period of ten to fifteen minutes after fertilization in which time the ectoplasmic layer forms. If the eggs are packed together by centrifuging after the ectoplasm is formed they do not stick together very readily. Eggs that were fused in pairs either spontaneously or by centrifuging after removal of the membranes were followed in about 300 cases. In most of these, double blastulae were formed which later separated, giving two normal embryos. In some, double gastrulae were produced. One normal giant was obtained.

In another way (essentially that of Driesch) the eggs were made

to stick together by placing them for two to five minutes in Ca-free sea water at various times after the formation of the ectoplasmic layer and then packing them together by gently centrifuging immediately after return to ordinary sea water. By this method three normal giants were obtained. The Ca-free sea water treatment and subsequent centrifuging was also applied to eggs in the two-, four- and eight-cell stages. Two normal giants were obtained from eggs so treated in the two-cell stage and 2 more from eggs treated in the four-cell stage out of more than 200 fused eggs that were isolated.

Eggs were handled individually on cinema film according to the method of Hörstadius. Two normal giants were obtained; one from a pair of eggs fused in the two-cell stage and another from a pair fused in the four-cell stage.

The eggs were also handled individually and fused in capillary tubes. A number of capillary tubes sealed at one end are prepared

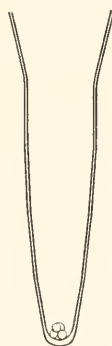


FIG. 1. Capillary tube for fusion of eggs.

(Fig. 1). The sealed end should have a rounded bottom obtained by blowing as the end is sealed off. About $\frac{3}{4}$ inch is a convenient length of tube. The tubes are first filled with Ca-free sea water by driving them down in a centrifuge tube containing that solution. They are then mounted vertically in a vial in a Stender dish containing Ca-free sea water so that their open ends are immersed. The eggs are first washed in Ca-free sea water and then, immediately, two eggs are placed in each capillary tube. Into some tubes only a single egg is inserted to serve as a control. The tubes are then transferred to a centrifuge tube containing ordinary sea water and are run at about 2,000 r.p.m. for 15 seconds to 3 minutes. The eggs are thus driven to the bottom of the capillary tube where they may fuse if the tube

is of the proper size and shape. During this time the ordinary sea water gradually diffuses into the capillary tube. If the eggs are allowed to remain in the tube they may develop into swimming blastulae. It is preferable, however, to remove them at an early stage by

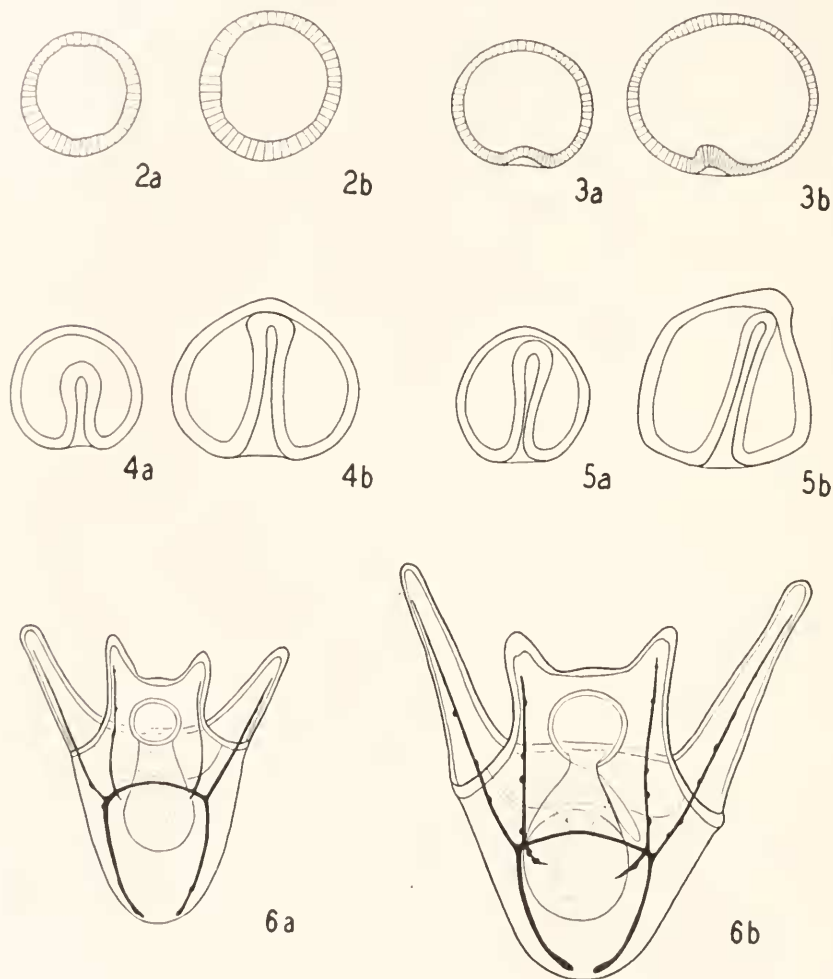


PLATE I

Giants from fused eggs and normal embryos of *Lytechinus*, drawn from photographs of living embryos.

- FIGS. 2a and b. Normal and giant embryos in blastula stage.
 FIGS. 3a and b. Normal and giant embryos in beginning gastrula stage.
 FIGS. 4a and b. Normal embryo about two-thirds gastrulated, giant embryo completely gastrulated.
 FIGS. 5a and b. Normal embryo completely gastrulated, giant embryo in prism stage.
 FIGS. 6a and b. Normal and giant embryo in pluteus stage.

breaking off the sealed end and inverting it in a dish of sea water. The eggs will then slowly drop out. Four normal giants were obtained from more than 150 fused eggs that were followed. Two of them were from eggs fused in the one-celled stage and 2 from eggs fused in the two-cell stage.

A total of 14 normal giants were thus obtained. Of these 10 developed faster than the normal control eggs and 4 developed at the same rate as the controls. The results are presented in Table I. The sea urchins used in these experiments were *Sphaerechinus granularis* and *Echinus microtuberculatus* at Naples and *Strongylocentrotus purpuratus* and *Lytechinus* at Corona del Mar, California. The material used and the method of treatment seemed to have no particular relation to the results.

The temperature was not controlled but single eggs treated in the same manner as the fused eggs were isolated in the same dish with the latter. These control eggs are listed with the giant eggs in the table. In four cases (Nos. 4, 7, 8, and 12) the giant and control embryos were found to be developing at the same rate. In the others the giants showed a faster rate of development. The difference in the rate of development is seen at the end of gastrulation and becomes more marked in the later stages. Thus in No. 11 the giant embryo is in the prism stage at a time when the control is still in the gastrula stage; in Nos. 6, 11, and 13 the giants are in the beginning pluteus stage while the corresponding controls are in the prism stage; and in Nos. 1, 3, 6, 11, 13, and 14 the giants are in the pluteus stage while the corresponding controls are entering that stage.

The giant embryos followed in these experiments were those that appeared normal throughout development. Those that showed two invaginations were discarded although they might sometimes give normal plutei according to Bierens de Haan (1913c) and Goldfarb (1917). Figures 2 to 6 show some of the giant embryos obtained. In Fig. 4b the giant embryo is a finished gastrula while the control embryo shown in Fig. 4a is about two-thirds gastrulated. Another giant embryo (Fig. 5b) is in the prism stage while the control embryo (Fig. 5a) is in the gastrula stage. In Figs. 6a and b a normal and a giant pluteus at $3\frac{1}{2}$ days are shown. The giant embryo entered the pluteus stage at 64 hours after fertilization and the normal at 82 hours.

The evidence shows then that the giant embryos develop more rapidly than the normal. They complete gastrulation sooner than the controls and the difference in rate of development increases as development continues. The data is not extensive enough to decide whether they both begin gastrulation at the same time, a point that would be of some interest to determine.

TABLE I

The time (in hours) at which various developmental stages of the giant and normal embryos are attained.

	Beginning Gastrula	Completed Gastrula	Prism Stage	Beginning Pluteus	Pluteus
1. <i>Spharechinus</i> :					
Giant.....	—	22	—	—	50
Control.....	—	25	—	50	62
2. <i>Echinus</i> :					
Giant.....	—	23	—	41	—
Control.....	—	25	41	—	—
3. ———					
Giant.....	16	19	—	50	58
Control.....	16	21	—	58	65
4. ———					
Giant.....	—	25	—	—	—
Control.....	—	25	—	—	—
5. <i>Strongylocentrotus</i> :					
Giant.....	26	30	—	—	—
Control.....	25	34	—	—	—
6. ———					
Giant.....	21	26	—	55	66
Control.....	21	29	55	66	79
7. <i>Lytechinus</i> :					
Giant.....	—	24	—	—	75
Control.....	—	24	—	—	75
8. ———					
Giant.....	19	24	—	—	—
Control.....	19	24	—	—	—
9. ———					
Giant.....	23	28	—	—	—
Control.....	25	32	—	—	—
10. ———					
Giant.....	25	30	—	—	—
Control.....	25	33	—	—	—
11. ———					
Giant.....	—	—	34	44	60
Control.....	—	34	44	60	—
12. ———					
Giant.....	—	30	41	—	—
Control.....	—	28	41	—	—
13. ———					
Giant.....	21	26	35	45	64
Control.....	22	30	45	64	82
14. ———					
Giant.....	—	28	39	—	65
Control.....	—	29	41	65	80

Discussion

The more rapid development of the giant embryos from fused eggs may be interpreted on the same basis as the slower development of the dwarf embryos from isolated blastomeres; namely, that twice

as much energy is available per unit time in the giant embryo, but less than twice the amount of energy is required for gastrulation and other form changes. The giant embryos thus proceed faster in development than the normal embryos.

The slower development of the half-sized embryos has been noted by Morgan (1895, 1903), Driesch (1900, 1908), Hörstadius (1928) and Tyler (1933) for the case of the sea urchin; by Morgan (1901) and Driesch (1903) in *Amphioxus*; by Spemann and Falkenberg (1919) and Fankhauser (1930) in *Triton*; by Tyler (1930) in *Chaetopterus*.

To interpret this slower development it has been assumed that a regulation process (whatever that might be) is involved in the formation of a whole embryo from an isolated blastomere and that the regulation requires time. Also to account for the still slower development of the embryos from $\frac{1}{4}$ -blastomeres (Driesch, 1900), it must be assumed that they require even more time for regulation. But when a single embryo is produced from two eggs there certainly must be as much regulation involved as when two embryos are produced from a single egg. On that basis we should expect the giant embryos to develop slower than the normal. Driesch (1900), however, reported that the giant embryo develops as fast as the normal, and the present results show that an even faster rate of development is obtained. Another argument against the assumption that a time interval for regulation accounts for the slower development of the dwarf embryos was presented by Spemann and Falkenberg (1919). They found that in an unequally constricted egg the smaller fragment develops slower than the larger, both being slower than the whole egg. The difference was found to increase during development and was greater than the amount of time that they would care to assume for regulation. They concluded that the factors causing the slower development must be associated with the smaller size of the embryo.

The faster development of the giant embryos from fused eggs has a parallel in the faster development of giant limbs from fused limb-buds reported by Filatow (1932). Filatow grafted a limb-bud of one embryo on to that of another embryo in the same stage. The normal giant limb that developed was considerably further advanced in differentiation than the normal limb of the other side at the times of examination. Although this result in a general way fits in very well with the views expressed here, it is perhaps at present inadvisable to push the parallelism too far.

Another experiment that bears more directly on this work is that of Spemann and Bautzmann (1927). They produced giant and dwarf embryos by cutting two beginning gastrulae of *Triton* parasagittally,

one to the left, the other to the right of the mid-line, and fused the two larger pieces together as well as the two smaller pieces. In cases in which both fused embryos developed normally, the giant embryo developed markedly faster than the dwarf embryo. It is of interest to note that they say in this connection:—"Das gilt nun ganz allgemein und hängt nicht etwa mit irgendeiner grösseren Schädigung des kleineren Keimes zusammen oder mit einer grösseren Schwierigkeit, die er bei der Regulation zu überwinden gehabt hätte, sondern einfach mit seiner geringeren Grösse und den damit wohl gegebenen grösseren Widerständen bei all den Verlagerungen und Faltungen, mit denen die Entwicklung verbunden ist." This greater resistance in the displacements and foldings of development of which Spemann and Bautzmann speak is one of the main assumptions of the theory developed here (see p. 1) and presented in the earlier work (Tyler, 1933). It is essentially what is meant by the statement (Tyler, 1933, p. 156) that "when similar changes in shape are produced, more work is performed in the case of two half-embryos than in one whole embryo." It is interesting that Spemann and Bautzmann also find this a reasonable assumption to make. It would, however, be well to obtain further justification for this assumption, and a mathematical attempt to do this on purely mechanical considerations is now in progress.

The purpose of studying the energetics of development is to obtain information that would be of value in determining the causes of the form changes. Such information is of value for the problem of differentiation in the same way that a knowledge of the energetics of muscular contraction is of importance in determining the chemical reactions responsible for that process. In the case of differentiation, however, it has been contended that no energy is required for the form changes (Needham, 1932). This conclusion is drawn from the measurements of Meyerhof (1911) and of Shearer (1922), showing a constant ratio of heat production to oxygen consumption throughout development. It was assumed that if energy were required for the form changes then the heat evolved per mole of oxygen consumed should decrease at times when work was being performed. Among the objections that may be raised against this point of view it may be pointed out that heat bears no labels—the energy may have been very useful in producing the form changes before it appeared as heat. There is no more reason for expecting the energy of differentiation to be stored up in the embryo than that it should be stored up in plastic deformation of non-living objects. We conclude from the work on dwarf embryos (Tyler, 1933) and on the giant embryos that energy is required for the form changes. As was pointed out in the

previous paper, the reason for the indirect method of attack is that we may recognize at least two other processes in development, namely maintenance and growth, which may require energy and which it was necessary to eliminate as factors in the result. The problem of obtaining a quantitative estimate of the energy required for differentiation may now be attacked and a number of methods suggest themselves. Among them the effect of temperature on the rate of development and on the rate of oxygen consumption is being investigated since if maintenance, growth, and differentiation should be found to have different temperature coefficients it would help in the analysis of the problem.

A question that has troubled embryologists for some time is why embryos from $\frac{1}{8}$ -blastomere may develop to the gastrula stage and then stop. As a possible explanation of the failure to develop further, it may be suggested that the tiny embryos do not have enough energy left to go on, but expend it all on the relatively increased work of gastrulation and on the accompanying maintenance and growth requirements.

Summary

1. Giant embryos produced by the fusion of two fertilized eggs develop more rapidly than normal sized embryos.
2. The bearing of this result on the energetics of differentiation is discussed.
3. A new method of fusing eggs is described.

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